Amino Acid Pools and the Role of PheRS in the Fidelity of Translation

A Senior Honors Thesis

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by

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Introduction

The central dogma of genetics states that deoxyribonucleic acid (DNA) encodes ribonucleic acid (RNA) through the process of transcription, which in turn encodes proteins through the process of translation.

$$\text{DNA} \overset{\text{transcription}}{\leftrightarrow} \text{RNA} \overset{\text{translation}}{\rightarrow} \text{protein}$$

Faithful translation of messenger RNA (mRNA) into protein denotes a crucial step in gene expression. Translation is an inherently accurate process with a proposed error rate of 10^{-4} (Loftfield and Vanderjagt, 1972). Two primary points determine the fidelity of translation during protein synthesis: aminoacylation of transfer RNA (tRNA) with the cognate amino acid and accurate selection of aminoacyl-tRNAs (aa-tRNAs) by the ribosome. The aminoacylation of tRNA takes place through a universally conserved two-step mechanism catalyzed by aminoacyl-tRNA synthetases (aaRSs). The amino acid is first activated with adenosine triphosphate (ATP) to form an aminoacyl-adenylate intermediate accompanied by the release of inorganic pyrophosphate (PP_i) (Equation 1). The activated amino acid is then transferred to the 3' end of the tRNA to form the amino acid-tRNA (aa-tRNA) pair; this step is accompanied by the release of adenosine monophosphate (AMP) (Equation 2).

$$\text{amino acid} + \text{ATP} + \text{aaRS} \leftrightarrow \text{aaRS}\bullet\text{aa-AMP} + PP_i \quad (1)$$

$$\text{aaRS}\bullet\text{aa-AMP} + \text{tRNA} \leftrightarrow \text{aaRS} + \text{aa-tRNA} + \text{AMP} \quad (2)$$

Aminoacyl-tRNA synthetase enzymes are generally specific for one amino acid, and they are divided into two classes based on differences in the structural topology of their active sites (Eriani *et al*, 1990). The two classes also vary in their overall structure and in the precise manner in which they carry out aminoacylation. Class I aaRSs are generally monomeric enzymes with

active sites characterized by a Rossmann-nucleotide-binding fold. They approach tRNA molecules from the minor groove of the tRNA acceptor stem and aminoacylate the terminal adenosine (of the CCA-3' terminal acceptor stem) at the 2'-OH position. In contrast, class II synthetases are typically multimeric enzymes whose active sites contain an antiparallel β-fold. They approach tRNAs from the major groove and charge the terminal adenosine at the 3'-OH position (Sprinzl and Cramer, 1975; Eriani *et al*, 1990).

In order to ensure accurate aminoacylation, aaRSs must correctly pair amino acids with the cognate tRNA molecule. The diverse combination of bases in tRNA molecules ensures that aaRSs specifically select cognate tRNA molecules for charging (Ebel *et al*, 1973). The acceptor stem of the tRNA molecule and the anticodon sequence play prominent roles in recognition of the appropriate tRNA by the synthetase. Generally, aaRSs achieve amino acid specificity by preferentially binding cognate over non-cognate amino acids. During the activation step, the active site of the synthetase enzyme preferentially selects for the cognate amino acid due to differences in the side chain binding energies of the substrate (Favorova, 1984).

Several factors affect whether the cognate amino acid is selected including the similarity of the cognate amino acid to other non-cognate amino acids and the presence of competing amino acids in the cell (Favorova, 1984). The variability of functional groups displayed by the twenty naturally occurring amino acids is relatively low; hence, amino acid discrimination by the aaRSs is not always accurate. When the cognate amino acid displays high structural similarity to another noncognate amino acid, the probability for misactivation and subsequent mischarging becomes higher. For example, phenylalanine (Phe) and tyrosine (Tyr) differ only by the presence of an additional hydroxyl group on tyrosine (see Figure 1). It has been shown that phenylalanyl-tRNA synthetase (PheRS), the aaRS responsible for charging $tRNA^{Phe}$ with

phenylalanine, may misactivate tyrosine (Roy *et al*, 2004). In such cases, additional editing activity may be necessary to ensure accurate aminoacylation and to prevent the incorporation of the wrong amino acid during protein synthesis.

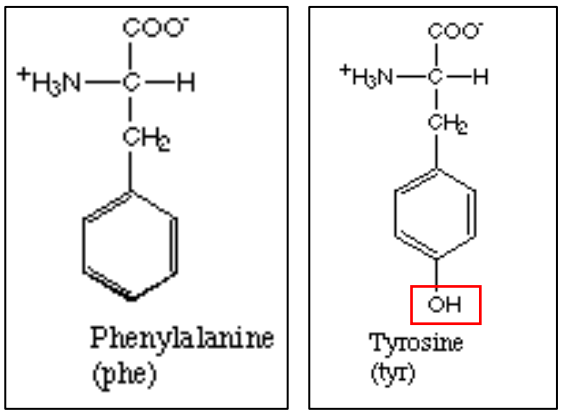

Fig 1: Chemical structures of the amino acids phenylalanine and tyrosine.

Editing by aaRSs

Although the innate specificity for recognition of cognate amino acid-tRNA pairs is often sufficient to ensure accurate aminoacylation, additional editing by the synthetase may be necessary if the cognate amino acid displays high structural similarity to other noncognate amino acids. AaRSs may possess pre- or post- transfer editing capabilities, thereby preventing the incorrect amino acid from being transferred to the ribosome and incorporated into the nascent polypeptide chain. Pre-transfer editing occurs by hydrolysis of the noncognate amino acid-AMP pair:

amino acid (aa) + ATP + aaRS ↔ aaRS • aa-AMP + PPi

Pre-transfer editing may occur by active site hydrolysis of the noncognate aminoacyl-adenylate (aa-AMP) prior to release from the active site; by selective release whereby the noncognate aa-AMP is expelled into solution where it can subsequently be hydrolyzed; or by translocation of

the aa-AMP to the editing site and ensuing hydrolysis of the noncognate aa-AMP at the editing site (Ling *et al*, 2009). Together, these mechanisms comprise pre-transfer editing.

Post-transfer editing occurs when the covalent bond between the noncognate amino acid-tRNA pair is hydrolyzed:

aaRS•aa-AMP + tRNA ↔ aaRS•aa-tRNA + AMP ↔ aaRS + aa-tRNA + AMP

Editing before release of the noncognate pair

The exact mechanism of how mischarged tRNAs move from the active site to the editing site remains unclear; however, it has been proposed that the aminoacylated 3' end of the tRNA translocates to the editing site while the rest of the tRNA molecule remains attached to the synthetase (Ling *et al*, 2009). Nucleophilic attack by water then enables hydrolysis of the aa-tRNA mismatch.

While some aaRs, such as alanyl-tRNA synthetase (AlaRS) and PheRS, rely on editing domains appended to the catalytic core of the aaRS, other synthetases may edit via discrete, unattached editing proteins or via binary complexes composed of one aaRS and a second protein factor (Hausmann and Ibba, 2008). For example, eubacterial prolyl-tRNA synthetase (ProRS), which is known to misactivate $tRNA^{Pro}$ with alanine and cysteine, forms a stable, ternary complex between ProRS, $tRNA^{Pro}$, and Ybak, a general tRNA binding protein (An and Musier-Forsyth, 2005). This ternary complex is required to hydrolyze mischarged Cys-$tRNA^{Pro}$ both *in vitro* and *in vivo* (Hausmann and Ibba, 2008). Ybak in association with ProRS may also assist in preventing the formation of mischarged Cys-$tRNA^{Pro}$, thereby further guarding against amino acid mis-incorporation during protein synthesis. AaRS editing activities are numerous, and our

current understanding of the diversity of aaRS editing capabilities may only capture a narrow fraction of the true complexity of these mechanisms.

The editing abilities of aaRSs augment the fidelity of translation, because editing prevents the accumulation of mischarged tRNA molecules and diminishes the potential for mis-incorporation of amino acids during protein synthesis. Studies of alanyl-tRNA synthetase have demonstrated that the editing motif of the enzyme can provide an independent determinant for alanyl-tRNA recognition (Beebe *et al*, 2008). This demonstrates that aaRSs may possess distinct motifs to facilitate cognate amino acid-tRNA pairing during both the aminoacylation step and the editing step. These distinct motifs add to both the complexity and the integrity of the enzymes.

Phenylalanyl-tRNA Synthetase: Structure, Function, and Editing

Phenylalanyl-tRNA synthetase (PheRS), the enzyme responsible for charging $tRNA^{Phe}$ with phenylalanine, is a member of the class II aaRSs. This classification is based on the structural topology of its active site, which is composed of a seven-stranded antiparallel β-sheet flanked by four alpha helices (Eriani *et al*, 1990). The structure of PheRS, which has been elucidated using X-ray crystallography, is one of the largest and most complex of the aaRSs (see Figure 2). Similar to other class II synthetases, PheRS is a multimeric enzyme: it is heterotetrameric, consisting of two α/β-heterodimers ($(\alpha\beta)_2$). One $tRNA^{Phe}$ molecule interacts with all four of the domains, thus accounting for the heterotetrameric structure (Goldgur *et al*, 1997; Lechler and Kreutzer, 1997). The acceptor stem and the 3'-CCA end of $tRNA^{Phe}$ interact with the active site in the α-subunit and with the N terminal end of the β-subunit of the same αβ heterodimer. The anticodon loop of $tRNA^{Phe}$ interacts with the β-subunit of the second αβ heterodimer, and the α-subunit of the second αβ heterodimer approaches the variable stem of the tRNA molecule.

While PheRS is structurally related to the class II aaRSs, enzymatically it resembles the class I enzymes in that it aminoacylates the 2'-OH of the terminal adenosine residue of the 3'-CCA end of $tRNA^{Phe}$ (Sprinzl and Cramer, 1975). Thus, PheRS possesses properties of both class I and class II synthetases.

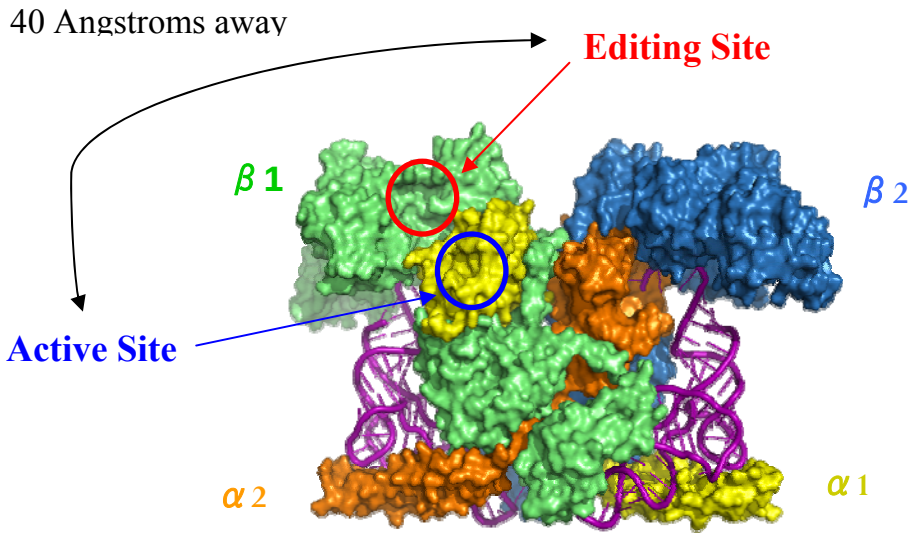

Fig 2: Structure of *Thermus thermophilus* PheRS, Goldgur *et al*, 1997.

The active site of the PheRS enzyme maps to the α subunit and the editing site maps to the B3/B4 domain of the β subunit; the two sites are separated by approximately 40 Angstroms (Roy *et al*, 2004). The active site is characterized by a deep, phenylalanine binding pocket (Reshetnikova *et al*, 1999). The bottom of the binding pocket is parallel to the phenylalanine substrate and is covered by glycine residues. Hydrophobic residues cover one wall of the pocket as well as the top surface of the pocket. Another wall of the pocket is composed of hydrophilic amino acid residues capable of participating in hydrogen bonding. The anisotropic distribution of hydrophobic and hydrophilic residues in the binding pocket and the relative depth of the pocket allow for stable and specific binding of phenylalanine and formation of phenylalanyl-adenylate (Phe-AMP) (Reshetnikova *et al*, 1999).

Specific recognition of phenylalanine from the pool of amino acids available in the cell is facilitated by the phenylalanine residues (Pheα-258, Pheα-260) in the binding pocket (Reshetnikova *et al*, 1999). Interactions between these residues and the incoming phenylalanine substrate are energetically favorable, because they lead to the formation of a stable network of interactions with "edge-to-face" contact between the phenylalanine residues (Burley and Petsko, 1985). Since this network of aromatic-to-aromatic interactions drives binding by PheRS, out of the pool of the twenty naturally occurring amino acids, only the aromatic amino acids (phenylalanine, tyrosine, and tryptophan) are candidates for binding (see Figure 3). The bulky side chain of tryptophan prohibits its binding in the active site. While tyrosine differs from phenylalanine only by the addition of a hydroxyl group on the phenyl ring, the hydrophobic residues in the binding pocket of PheRS do not favor binding to tyrosine, and facilitate the enzyme's innate selectivity toward phenylalanine. However, as previously noted, PheRS does not always successfully discriminate between these two amino acids and mis-activation of tyrosine and subsequent synthesis of Tyr-tRNA^{Phe} may occur (Roy *et al*, 2004).

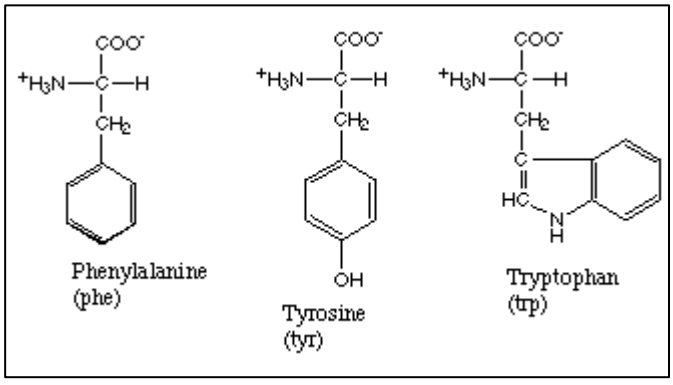

Fig 3: Chemical structures of the aromatic amino acids.

When tRNA^{Phe} is mischarged with tyrosine to form Tyr-tRNA^{Phe}, the post-transfer editing ability of PheRS may correct the error. Translocation of the aminoacylated 3'-end of the tRNA from the active site to the editing site enables hydrolysis of the noncognate amino acid-tRNA

pair before release into the cytosol (Roy *et al*, 2004). Editing site residues position the noncognate substrate (Tyr-tRNA^{Phe}) for nucleophilic attack by water. Both structural data and tyrosine-dependent ATP hydrolysis enhanced by tRNA^{Phe} provide evidence for a preferential post-transfer editing pathway (Kotik-Kogan *et al*, 2005). This post-transfer editing activity corrects errors occurring during amino acid selection and prevents Tyr-tRNA^{Phe} from accumulating in the cell, thereby ultimately enhancing the fidelity of translation. Moreover, during translation, eubacterial elongation factor Tu (Ef-Tu) recognizes Tyr-tRNA^{Phe} as efficiently as cognate Phe-tRNA^{Phe}, and the ribosome lacks the ability to distinguish between the two (Ling *et al*, 2007). This suggests that the post-transfer editing activity of PheRS is the major mechanism by which tyrosine mis-incorporation is prevented. Thus the importance of editing by *E. coli* PheRS cannot be overstated; it is one of the crucial factors for ensuring accurate protein synthesis and for preventing tyrosine mis-incorporation at phenylalanine codons.

Studies of *Escherichia coli* have demonstrated that replacement of key residues at the editing site abolishes editing activity by PheRS (Roy *et al*, 2004). Substitutions in the B3/B4 domain at the entrance of the proposed editing site (βT354W, βA356W) or within the binding pocket itself (βH256A or L, βE334A) severely inhibit editing by PheRS (Roy *et al*, 2004). This allows for the mismatch Tyr-tRNA^{Phe} pair to be stably synthesized under both *in vivo* and *in vitro* conditions, promoting mis-incorporation of tyrosine during protein synthesis, and hence degrades the fidelity of translation.

Questions to be addressed and experimental design overview

Although the mechanism of hydrolysis of Tyr-tRNA^{Phe} has been examined, the impact of editing at the cellular level remains unclear. When the concentrations of tyrosine (Tyr) and phenylalanine (Phe) in the cell are equal, the rate of misacylation is surprisingly low: the proportion of tRNA^{Phe} that is misacylated with tyrosine is approximately 1:8000 (1 Tyr-tRNA^{Phe}:8000 Phe-tRNA^{Phe}). Such a low rate of error is presumed to be negligible and does not impact the cells' growth rate under normal conditions in the laboratory (i.e. when the cells are not experiencing physiological stress or nutritional deficiencies). Hence, the purpose of the editing mechanism is uncertain.

Two possible functions of the editing mechanism are that editing serves an important role when cells are experiencing physiological stress and/or that the role of editing rises in prominence under a high concentration of tyrosine. In an environment with a relatively high concentration of tyrosine, mis-charging of tRNA^{Phe} with tyrosine by PheRS would be more likely. In cells with intact editing capabilities, the problem of mis-charging can be overcome by the editing activity of the PheRS enzyme. However, editing deficient cells accumulate Tyr-tRNA^{Phe} in the cytosol, and consequently mis-incorporate tyrosine in place of phenylalanine during protein synthesis (Roy *et al*, 2004).

Little is known about the conditions under which the cell contains an abundance of tyrosine or the need for editing in response to changes in amino acid pools. To assess how fluctuations in the concentrations of the amino acids tyrosine and phenylalanine impact the quality control mechanisms of *E. coli* PheRS, and hence impact the fidelity of translation and gene expression, growth of four strains of *E. coli* in varying concentrations of phenylalanine and tyrosine was assessed. Additionally, data is also lacking on how intracellular amino acid pools

fluctuate with amino acid availability in the extracellular environment. To address this question, samples of cells grown under different concentrations of tyrosine and phenylalanine were collected, and prepared for mass spectrometry analysis to quantify the intracellular amino acid pools[1].

Methods

Strains used

Four strains of *Escherichia coli* were used (see Figure 4 for a summary). The mutations of Mutants 0 and 7 are on plasmid borne genes. Mutant 0 is characterized by an alanine to glycine mutation (PheRSαA294G) in the active site of PheRS. This change results in a promiscuous active site which allows mutant 0 PheRS to accommodate tyrosine better than wild type *E. coli*, resulting in $tRNA^{Phe}$ charged with tyrosine. However, since the editing activity of the PheRS is conserved in mutant 0, the mismatch Tyr-$tRNA^{Phe}$ can be hydrolyzed by the post-transfer editing activity of PheRS to prevent its accumulation in the cell. In contrast, mutant 7, which possesses the same mutation of the active site as mutant 0, has an additional mutation in the editing site (PheRSαA294G/βA356W) which abolishes its editing capability. Since mutant 7 PheRS is editing deficient, it allows stable Tyr-$tRNA^{Phe}$ to be released from PheRS and to accumulate in the cell. This should lead to a decrease in the fidelity of translation, because in such circumstances, tyrosine can be mis-incorporated into the polypeptide chain in place of phenylalanine.

KA3 is a strain of wild type *E. coli* and is editing competent. KA4 contains the same mutation as mutant 0, only rather than being plasmid encoded, KA4 carries the mutation on its

[1] Although the samples were prepared for mass spectrometry analysis, at the time of graduation they had yet to be processed; thus, the results from the mass spectrometry were unavailable and are not included in this paper.

chromosome. A chromosomal mutant analogous to mutant 7 (PheRSαA294G/βA356W) is currently being developed, and hence growth of such a strain was not studied here.

	Strain	Mutation	Editing Deficient	Editing Intact
Plasmid	Mutant 0	PheRSαA294G		×
	Mutant 7	PheRSαA294G/βA356W	×	
Chromosomal	KA3	wild type		×
	KA4	PheRSαA294G		×

Fig 4: Summary of the strains used to establish growth curves.

Growth Curves

Growth curves of the mutant strains *E. coli* PheRSαA294G (Mut 0) PheRSαA294G/βA356W (Mut 7), KA3, and KA4 were established. Cultures were initially grown aerobically overnight at 37 ºC in 5 mL of LB liquid media. 1 mL of the overnight culture was then transferred to a 250-mL Erlenmeyer flask, prepared in triplicates. These cultures were grown aerobically at 37 ºC in 100 mL (total) of media in the 250-mL Erlenmeyer flasks. A minimal media (M9) base was used and supplemented with: ampicillin[2] (200 μg/mL), calcium chloride ($CaCl_2$, 0.1 mM), glucose (2 mg/mL) magnesium sulfate ($MgSO_4$, 1mM), a mixture of 18 amino acids (-tyrosine, -phenylalanine, 40 mg/L), and thiamine (1 μg/mL). Four growth conditions with alternating low (40 μg/mL) and high (200 μg/mL) concentrations of phenylalanine and tyrosine were established (see Figure 6). The cultures were sampled at one (for KA3 and KA4) or two (for Mut 0 and Mut 7) hour intervals. Growth was quantified spectrophotometrically as absorbance at 600 nm. IPTG (1mM, 0.2 mL) was added to the cultures with the plasmid encoded mutants (Mut 0 and Mut 7) to induce over expression of the plasmid-encoded PheRS when the growth reached an optical density (OD) of ~0.3.

[2] Ampicillin was only supplied in the media for Mutant 0 and Mutant 7, the strains carrying a plasmid which confers ampicillin resistance.

Component	Concentration
Ampicillin	200 μg/mL
$CaCl_2$	0.1 mM
Glucose	2 mg/mL
$MgSO_4$	1 mM
Mixture of 18 amino acids (–Phe, –Tyr)	40 μg/mL
Thiamine	1 μg/mL

Fig 5: Supplements for M9 base.

Condition	Phenylalanine Concentration	Phe, μg/mL	Tyrosine Concentration	Tyr, μg/mL
1	Low	40	Low	40
2	High	200	Low	40
3	Low	40	High	200
4	High	200	High	200

Fig 6: Growth conditions with variable concentrations of Phe and Tyr.

Preparation of the samples for mass spectrometry analysis

The experiments were repeated and 3.0 mL samples were collected for mass spectrometry analysis. The cells were collected, pelleted, washed with 0.1 M Tris-HCl buffer (pH 8.0, 2 × 0.5 mL), and stored at –80 ºC until processing.

The pelleted samples were resuspended in 500 μL 2% trifluoroacetic acid (TFA) and disrupted by sonication (3 × 10 sec). The homogenate was centrifuged, and the supernatant was transferred to a clean microcentrifuge tube. The pellet was re-extracted with an additional 200 μL 2% TFA and centrifuged; the supernatants were then pooled. Labeled phenylalanine (15 μg) and tyrosine (15 μg) were included in each microcentrifuge tube as internal standards. The pooled supernatants were placed on ice and extracted with ethyl acetate (3 × 700 μL). The supernatant was discarded and the aqueous phase was transferred to a clean microcentrifuge tube, dried using a speedvac (5 hrs total drying time) and stored at –80 ºC[3].

[3] At the time of graduation, the samples had not been processed; thus analysis of the samples is not included in this paper.

Results of growth curves (Figures 7-14)

The growth curves reflect the averages for triplicates of each strain under each growth condition, and the error bars reflect the standard deviations. The wider error bars observed for mutant 0 and mutant 7 (in comparison to KA3 and KA4) may reflect differences resulting from variations in the copy number of the plasmid in these two strains.

Although slight differences in growth were observed for mutant 0 and mutant 7 under the low Tyr/low Phe, low Tyr/high Phe, and high Tyr/high Phe conditions, these differences are not significant and may reflect differences in the starting ODs of the cultures. However, under the high Tyr/low Phe condition, mutant 7 (the editing deficient strain) exhibited a much longer lag phase relative to mutant 0 under the same condition (Figure 9). The lag phase was also longer than that observed under any of the other growth conditions. This extended lag period may reflect a greater disruption in the fidelity of translation resulting from the loss of editing by PheRS. It also suggests that the editing function of PheRS may be most important for cells growing in the presence of a relative abundance of tyrosine.

Virtually no differences in growth rate or pattern were observed for KA3 or KA4 under any of the conditions tested (Figures 10-13). Since both of these strains have intact editing activity by PheRS, the lack of a difference is not unexpected.

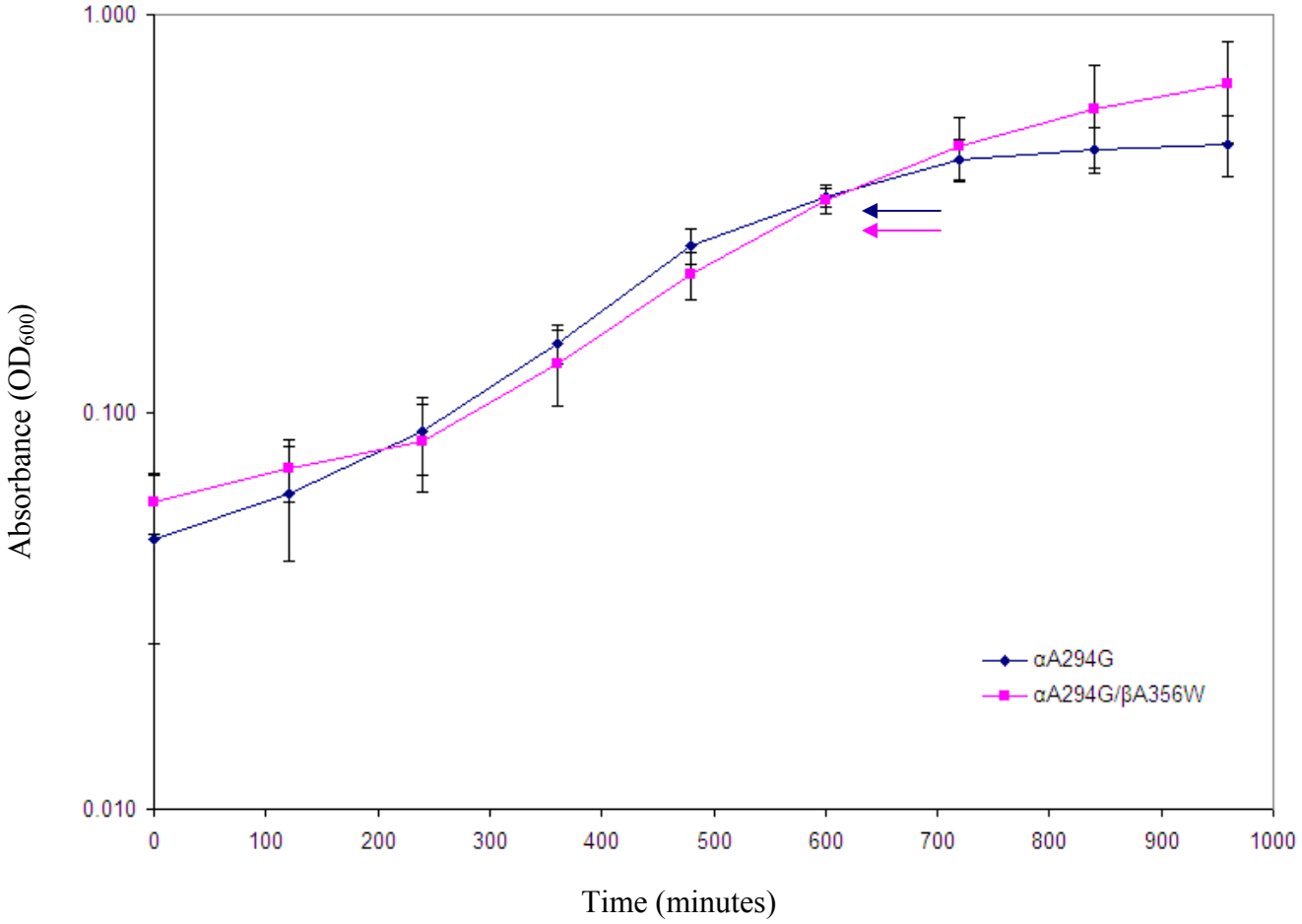

Fig 7: Mutant 0 (PheRSαA294G) and Mutant 7 (PheRSαA294G/βA356W), Low Tyr/Low Phe. The arrows (← for Mut 0 and ← for Mut 7) indicate the time point at which isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce over-expression of the plasmid encoded mutant PheRS.

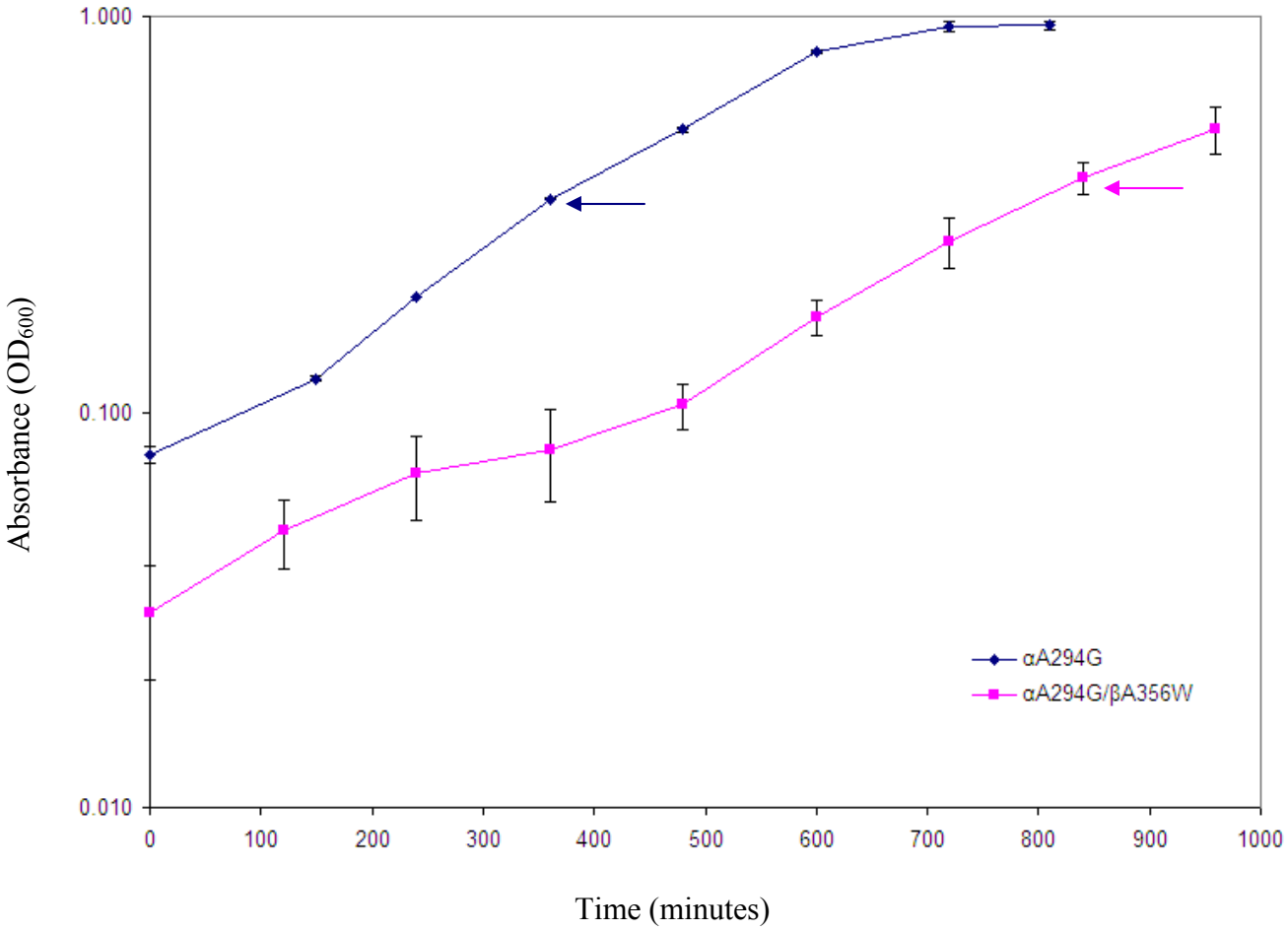

Fig 8: Mutant 0 (PheRSαA294G) and Mutant 7 (PheRSαA294G/βA356W), Low Tyr/High Phe. The arrows (← for Mut 0 and ← for Mut 7) indicate the time point at which isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce over-expression of the plasmid encoded mutant PheRS.

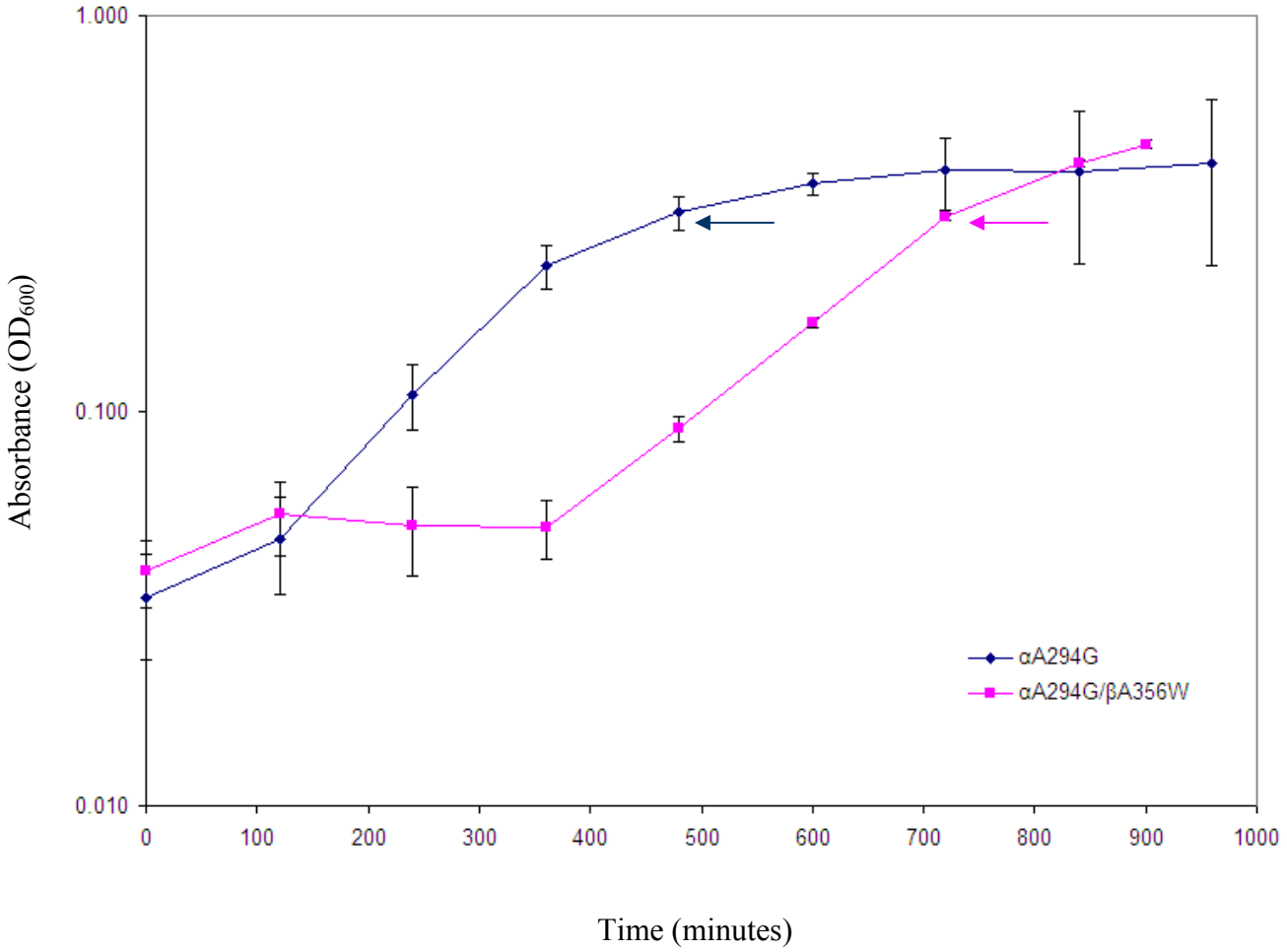

Fig 9: Mutant 0 (PheRSαA294G) and Mutant 7 (PheRSαA294G/βA356W), High Tyr/Low Phe. The arrows (← for Mut 0 and ← for Mut 7) indicate the time point at which isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce over-expression of the plasmid encoded mutant PheRS.

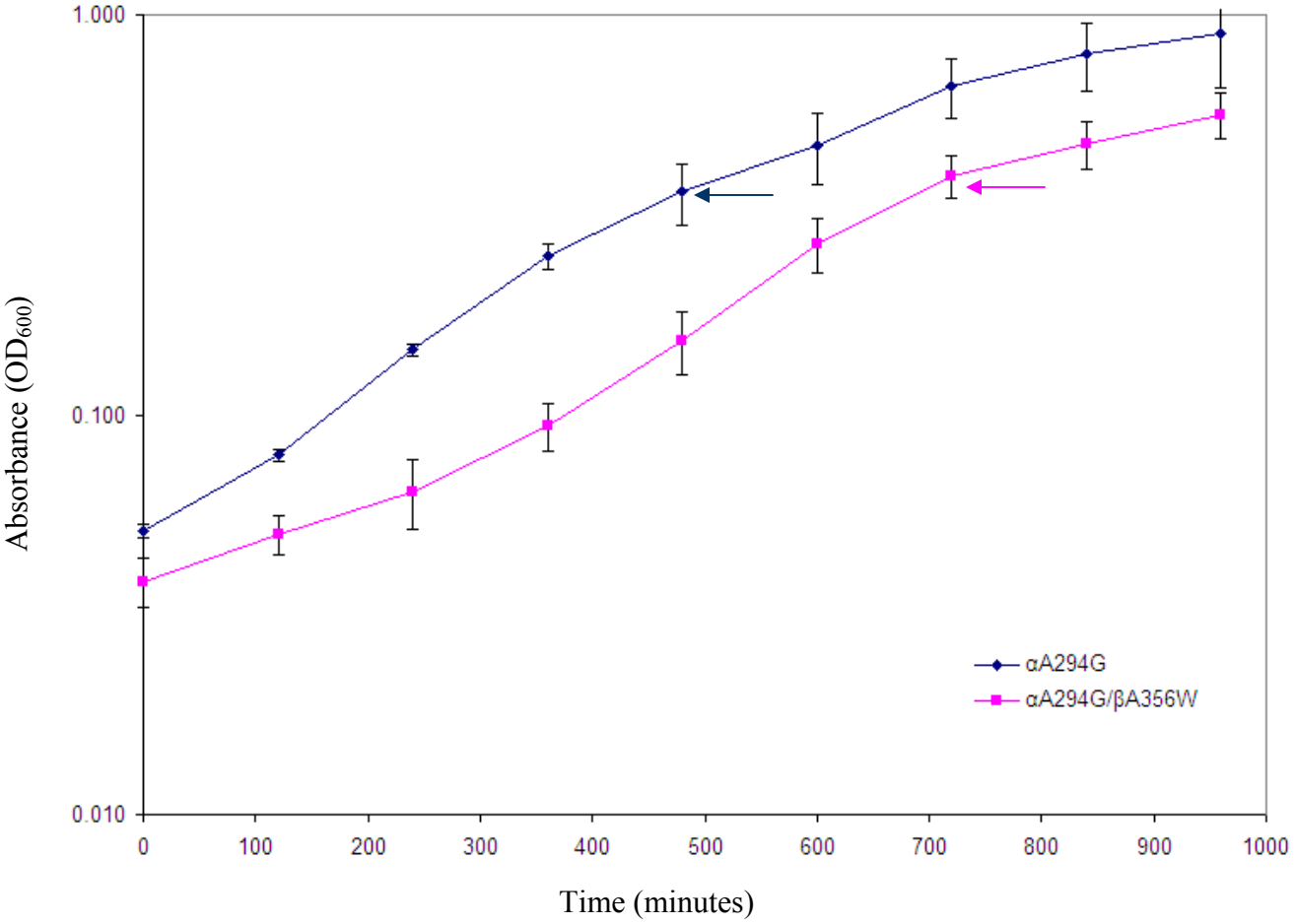

Fig 10: Mutant 0 (PheRSαA294G) and Mutant 7 (PheRSαA294G/βA356W), High Tyr/High Phe. The arrows (← for Mut 0 and ← for Mut 7) indicate the time point at which isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce over-expression of the plasmid encoded mutant PheRS.

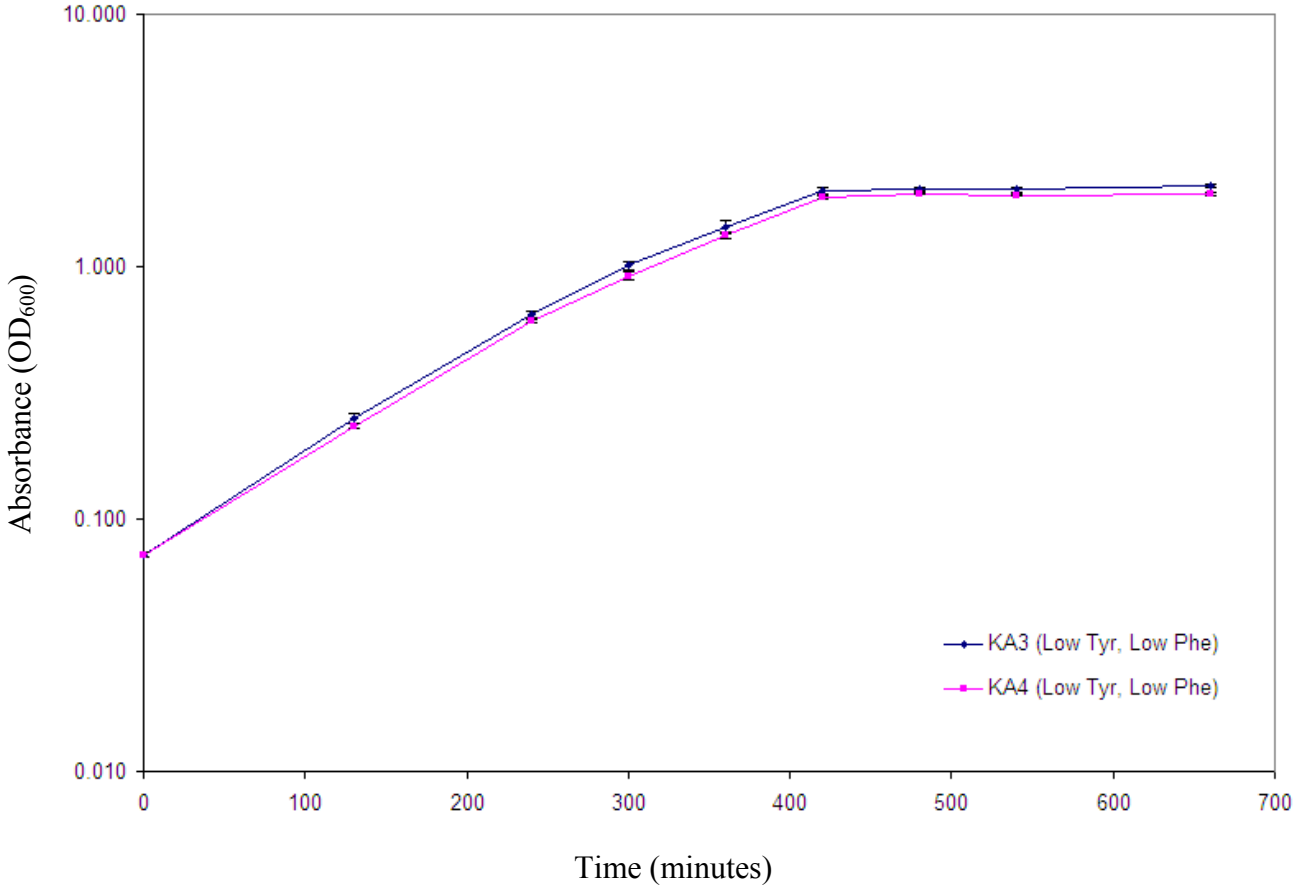

Fig 11: KA3 (wild type) and KA4 (PheRSαA294G), Low Tyr/Low Phe.

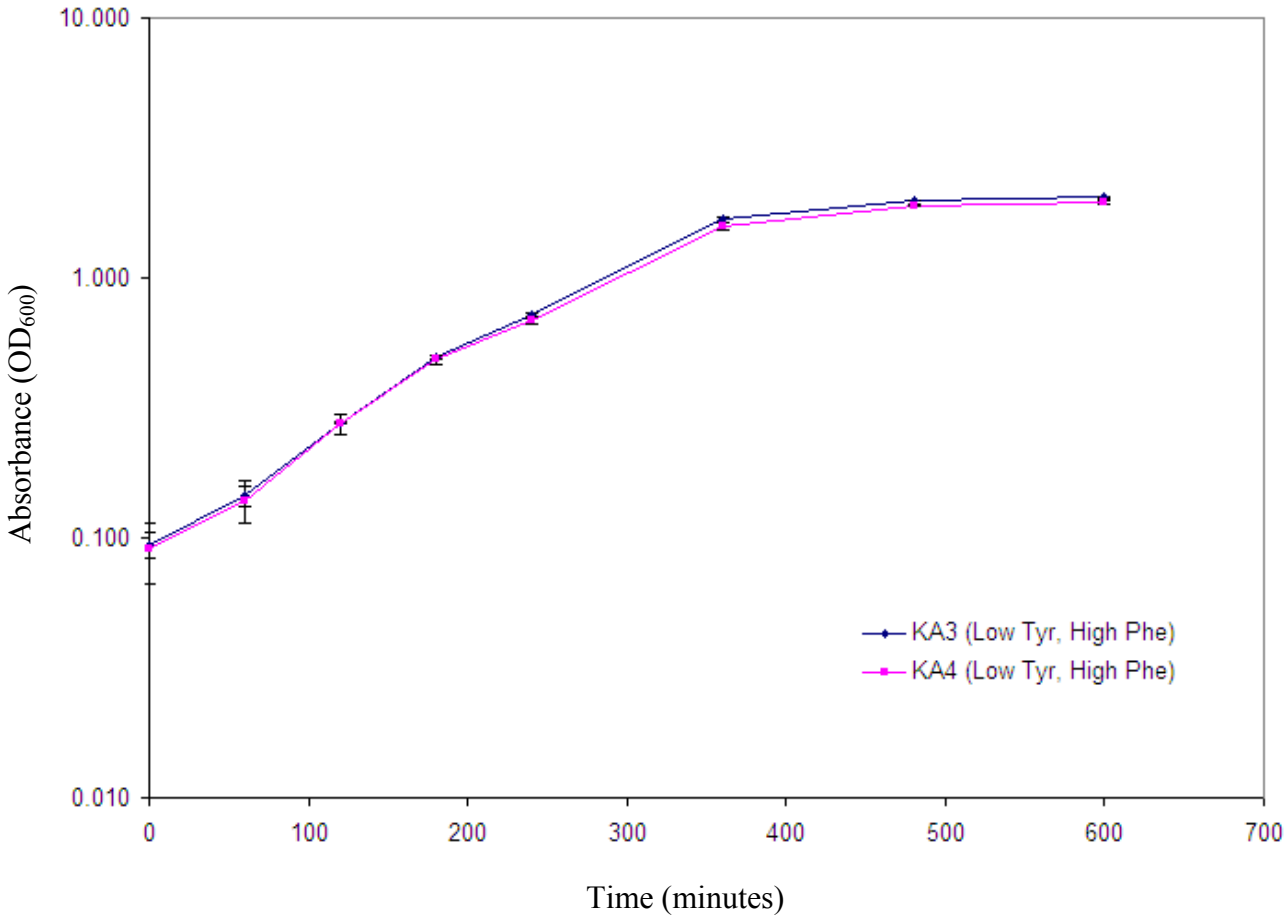

Fig 12: KA3 (wild type) and KA4 (PheRSαA294G), Low Tyr/High Phe.

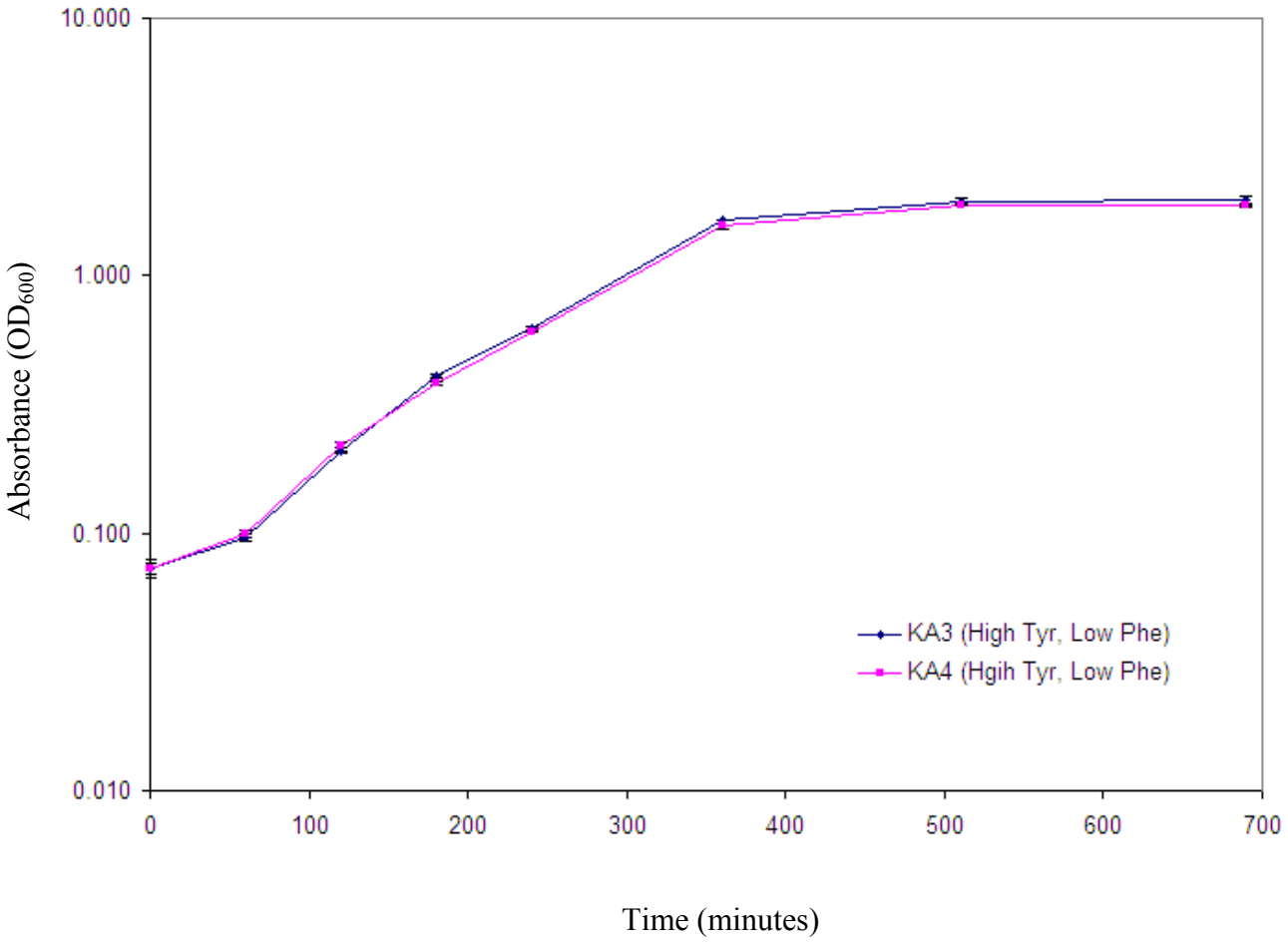

Fig 13: KA3 (wild type) and KA4 (PheRSαA294G), High Tyr/Low Phe.

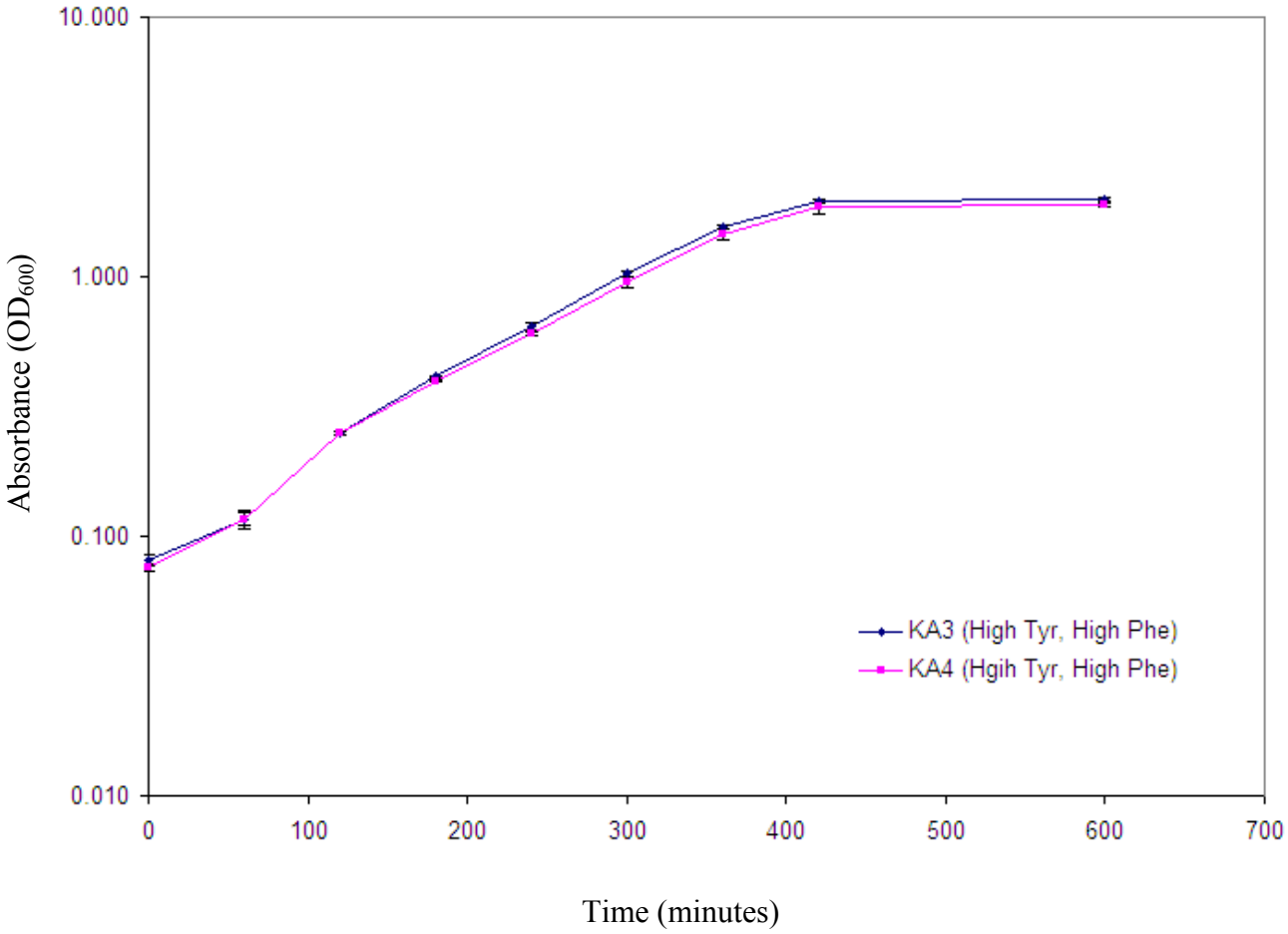

Fig 14: KA3 (wild type) and KA4 (PheRSαA294G), High Tyr/High Phe.

Discussion

The accurate coupling of cognate amino acids and tRNAs by aaRSs relies on the substrate specificity of the aaRS for both the amino acid and the tRNA and on the catalytic editing capability of the enzyme. Editing by *E. coli* PheRS ensures faithful translation of mRNA into protein. The results of these experiments indicate that the editing deficiency of PheRS may be most problematic for cell growth in stressful environments, such as in an environment where the relative concentration of tyrosine to phenylalanine is high. In such an environment, misactivation of tyrosine by PheRS and subsequent mischarging of $tRNA^{Phe}$ with tyrosine is more probable. The ensuing accumulation of Tyr-$tRNA^{Phe}$ in the cell can lead to mis-incorporations during protein synthesis and the production of misfolded and inactive proteins. Refolding and degrading misfolded proteins places an additional energy burden on the cell, and can be particularly detrimental when cells are initially exiting lag phase.

The extended lag period observed for the editing deficient strain (mutant 7, PheRSαA294G/βA356W) under the high Tyr/high Phe condition was the primary discrepancy observed in any of the growth curves. Since *E. coli* does not have enzymes capable of converting the excess tyrosine into phenylalanine, the possibility that the activation of such enzymes could account for the extended lag period is improbable (Underfriend and Cooper, 1951). Rather, the greatly extended lag period exhibited by the editing deficient strain relative to the editing competent strain under the same growth condition may be the result of a specific, unidentified stress response.

While the editing activity of aaRSs is one of the key determinants in the maintenance of faithful translation, resampling of the aminoacyl-tRNA product by the aaRS may occur. When the aminoacyl-tRNA is bound to EF-Tu*GTP to form a ternary complex, it may dissociate from

this complex, rebind to the aaRS, and undergo resampling via the editing pathway (Ling *et al*, 2009). In *in vitro* experiments, this leads to a tenfold reduction in the error rate during translation and serves as an additional regulator of accurate polypeptide synthesis (Ling *et al*, 2009). Under physiological conditions, wild type PheRS has also been shown to compete effectively with EF-Tu for free Tyr-tRNA^{Phe} and to hydrolyze the noncognate pairing (Ling *et al*, 2009). However, editing deficient mutant 7 PheRS would be unable to successfully hydrolyze Tyr-tRNA^{Phe} even during resampling; thus an editing deficiency can actually negatively impact faithful translation both during the initial aminoacylation and editing steps and during subsequent resampling prior to translation elongation.

Implications of the Study

The implications of this study are relevant to understanding diseases which result from errors in the editing pathways of aminoacyl-tRNA synthetases. Although no defect in the editing mechanism of an aaRS has been directly implicated in human pathology, it has been demonstrated that defective editing by AlaRS causes mice to exhibit a phenotype characterized by rough, unkempt fur, the loss of terminally differentiated Purkinje cells in the cerebellum, and severe ataxia (Lee *et al*, 2006). Denoted the sticky mutation (*sti*), the A734E change observed in the editing site of AlaRS of these mice results in stable synthesis of Gly-tRNA^{Ala} and Ser-tRNA^{Ala} resulting in mis-incorporations during translation (Lee *et al*, 2006). The resultant faulty, misfolded proteins disrupt the balance between the unfolded protein load and the endoplasmic reticulum (ER) folding machinery, ultimately triggering the unfolded protein response to reestablish homeostasis (Lee *et al*, 2006). The diseased phenotype resulting from the A734E mutation in the editing site of AlaRS was only observed in the Purkinje cells. Since Purkinje cells are terminally differentiated, they accumulate misfolded proteins more so than

other, non-differentiated cells; thus, a mutation in AlaRS – which degraded the fidelity of translation – had a greater impact on the Purkinje cells. This study illustrates how defective editing by an aaRS can be directly involved in neurological disease. Congruous editing defects in aaRSs may account for neurological pathology in humans.

The examination of intracellular amino acid pools also has implications for understanding how disrupting amino acid pools may lead to pathogenesis. Studies of the pool of free amino acids in mammalian mitochondria have indicated that they reflect the frequency of amino acids in mt-DNA encoded proteins (Ross-Inta *et al*, 2008). This seems to be the result of proteolytic degradation of mtDNA-encoded proteins, rather than the fulfillment of an amino acid profile to suit mitochondrial protein synthesis. Results from the Ross-Inta study suggest that the mitochondrial amino acid pool also differs markedly from the cytosolic pool, which reflects differences in the metabolic functions of the mitochondria and the cytosolic space; however, this conclusion is not definitive and other studies cite conflicting data. Quantification of intracellular and organellar (in the case of eukaryotes) amino acid pools will further the present understanding about their role in metabolism and how aberrations in amino acid pools may correlate with disease.

While aminoacyl-tRNA synthetases primarily participate in aminoacylation of tRNAs during the process of translation, they are also known to participate in a variety of other biological functions (Hausmann and Ibba, 2008; Ivanov *et al*, 2000; Martinis *et al*, 1999). AaRSs participate in the regulation of gene expression at various levels, including during transcription, mRNA processing, and translation. Tyrosyl-tRNA synthetase has been shown to have cytokine activity (Ivanov *et al*, 2000) while multi-protein complexes containing aaRSs have been implicated in apoptosis and viral assembly (Hausmann and Ibba, 2008). The modular

structure of PheRS also makes it an excellent candidate for participation in unconventional cellular processes. Studies of human PheRS have indicated that overexpression of the catalytic α-subunit of the enzyme may play a role in tumorigenic events during the development of myeloid leukemia (Rodova *et al*, 1999). Although not directly linked to the editing function of PheRS, this study demonstrates the importance of the intact $(\alpha\beta)_2$ heterodimer for maintenance of the cell cycle and normal cell differentiation. Future work will undoubtedly lead to novel discoveries about the diversity of aminoacyl-tRNA synthetase functions and their roles in maintaining normal cell physiology.

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